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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/048,244	08/27/2002	Donald K. Blumenthal II	0274-3858.1US	2667

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EXAMINER	
SODERQUIST, ARLEN	
ART UNIT	PAPER NUMBER
1743	

DATE MAILED: 04/28/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/048,244	BLUMENTHAL, DONALD K.	
	Examiner	Art Unit	
	Arlen Soderquist	1743	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *08 March 2005*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-27* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-27* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on March 8, 2005 has been entered.

2. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

3. Claims 1-2, 5-7, 9, 11, 15 and 21 are rejected under 35 U.S.C. 102(b) as being clearly anticipated by Odom (newly cited and applied). In the paper Odon discusses an apparent conformational change in phenylalanine transfer RNA that is associated with the peptidyl transferase reaction. Fluorescence techniques were used to detect changes in the conformation of $tRNA^{Phe}$ that may occur during the peptidyl transferase reaction in which the tRNA appears to move between binding sites on ribosomes. Such a conformational change may be a fundamental part of the translocation mechanism by which tRNA and mRNA are moved through ribosomes. Escherichia coli $tRNA^{Phe}$ was specifically labeled on acp^3U_{47} and s^4U_8 or at the D positions 16 and 20. The labeled tRNAs were bound to ribosomes as deacylated $tRNA^{Phe}$ or AcPhe-tRNA. Changes in fluorescence quantum yield and anisotropy were measured upon binding to the ribosomes and during the peptidyl transferase reaction. In one set of experiments ***non-radiative energy transfer was measured between a coumarin probe at position 16 or 20 and a fluorescein attached to acp^3U_{47} on the same $tRNA^{Phe}$ molecule***. The results indicate that the apparent distance between the probes increases during deacylation of AcPhe-tRNA as a result of peptide bond formation. All of the results are consistent with but in themselves do not conclusively establish that tRNA undergoes a conformational change as well as movement during the peptidyl transferase reaction. See the various tables and results and discussion sections. In particular page 933 discusses how the fluorescence of the coumarin label changes as the environment of the singly labeled substrate changes. Also pages 934-935 discuss the energy transfer and its use in determination of conformational changes in molecules by measuring

changes in the distance between the tow labels of the substrate.

4. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

5. Claims 1-27 are rejected under 35 U.S.C. 103(a) as being unpatentable over Blumenthal in view of Odom as explained above and Tyagi (either (WO 97/39008 or US 6,150,097, both newly cited and applied). In the paper Blumenthal reviews the development and characterization of fluorescently-labeled myosin light chain kinase calmodulin-binding domain peptides. The development and characterization of peptides based on the sequence of the calmodulin-binding domain of skeletal muscle myosin light-chain kinase which were labeled with the fluorescent reagent, acrylodan are described. The use of these fluorescently-labeled peptides to study various aspects of calmodulin-peptide interactions including binding affinity, stoichiometry, specificity, changes in peptide conformation, and thermal stability of the peptide-calmodulin complex is demonstrated. Page 46 discusses the preparation of analogs by replacing different amino acids within a natural peptide sequence to examine these properties. Blumenthal also teaches the formation of a library of peptides with different fluorescent labels. Page 46 also discusses the change in the acrylodan fluorophore as the environment changes and how this is useful in determining various properties. The peptides exhibit many of the salient features seen with calmodulin-target enzyme interactions. The fluorescently-labeled peptides should serve as useful models for studying calmodulin-target enzyme interactions at the molecular level.

Blumenthal does not teach the use of a substrate that is doubly labeled with labels that have at least a part of their energy transfer through non-radiative pathways.

Since the US Patent is a continuation of the application that resulted in the WO publication, only the US Patent will be discussed by reference the parts of the reference. In the patent Tyagi teaches detection of probes in nucleic acid hybridization using non-fluorescence resonance energy transfer (non- FRET) pairs of chromophores. Nucleic acid hybridization probes are described having a first conformation when not interacting with a target and a second conformation when interacting with a target, and having the ability to bring a label pair into touching contact in one conformation and not the other, are labeled with a non-FRET pair of chromophores and generate a fluorescent or absorbance signal. As opposed to FRET, quenching molecules and even other fluorophores can serve as efficient quenching moieties for fluorophores when attached to nucleic acid hybridization probes such that the fluorescing moiety and quenching moiety are in contact, even when the rules of FRET are violated. To demonstrate probes with "touching" pairs of a fluorophore with another fluorophore or quencher, where the pairs are not FRET pairs, fluorescence quenching efficiency was measured where Molecular Beacon probes were end-labeled with DABCYL at one end and one of 8 different fluorophores at the other end. DABCYL could quench the fluorescence of fluorescein, Lucifer Yellow, BIDIPY, eosine, erythrosine, tetramethylrhodamine, Texas Red, and coumarin. Effective fluorophore quenching also occurred in non-FRET pairs containing other quenchers, DABMI and Malachite Green, as well as appropriate (shorter wavelength) fluorophores such as coumarin. The utility of fluorophore-quencher combinations is demonstrated in a multiplex detection assay using 4 different nucleic acid targets. Columns 1-3 discuss the use of FRET labeled substrates in the prior art and notes that a disadvantage is related to the requirement for overlap between the labels in order to produce the desired affect. Columns 3-4 discuss the advantages of the non-FRET label pairs in that they do not require the overlap of FRET pairs and can therefore provide measurable results or enhancement even when FRET results are not possible.

It would have been obvious to one of skill in the art at the time of the invention to incorporate a double label selected from those taught by Odom or Tyagi in the Blumenthal substrates for their recognized ability to determine conformational changes and work in situation

that FRET label pairs or single labels are not able to provide that information as taught by Odom and Tyagi.

6. Claims 1-27 are rejected under 35 U.S.C. 103(a) as being unpatentable over Macala, Schultz (US 5,580,747) or Ventura in view of Blumenthal and Odom or Tyagi as explained above.

In the paper Macala teaches measurement of cAMP-dependent protein kinase activity using a fluorescent-labeled Kemptide. Traditional protein kinase assays include the use of ^{32}P-labeled ATP as phosphate donor and a substrate protein or peptide as phosphoreceptor. Since this approach has a number of drawbacks in addition to generating ionizing radiation, several non-isotopic methods have been developed. Although shown to reflect the activity of purified enzymes, none have been demonstrated to detect physiological changes in endogenous enzyme activity in cell homogenates. Studies were performed to examine the kinetics, reproducibility, and optimal assay conditions of a novel non-radioisotopic kinase assay that detects protein kinase A (PKA) activity by phosphorylation of the peptide substrate, Kemptide, covalently bound to a fluorescent molecule (fluorescamine-labeled Kemptide; f-Kemptide). Fluorescence was determined by spectrofluorometry with excitation at 568 nm and emission at 592 nm. Basal and agonist-induced PKA activities in epithelial cell homogenates were measured. The kinetics of f-Kemptide were similar to the standard radioisotopic method with intra-assay and inter-assay variations of 5.6 ± 0.8% and 14.3 ± 2.6%, respectively. Neither fluorescence quenching nor enhancing effects were found with consistent amounts of homogenate protein. Specific PKA activity was determined as the IP20-inhibitable fraction to account for nonspecific phosphorylation, perhaps due to S6 kinase or a similar enzyme. The basal activity of 38% of total PKA in A6 cells increased by 84% after exposure to vasopressin and by 58% after short exposure to forskolin. In T84 cells exposed to VIP there was a 360% increase over basal activity. These results show that f-Kemptide exhibits acceptable kinetics, and that the assay system can quantitatively and reproducibly measure basal and stimulated PKA activity in cell homogenates. Macala does not teach the substrate having two dyes attached or a library of compounds.

In the patent Shultz teaches a non-radioactive assay and purification of proteins, and particularly to the non-radioactive assay and purification of protein kinases, phosphatases and

protease by incubating the enzyme with a substrate modified peptide to form a product modified peptide under conditions where the enzyme is active. The product modified peptide and substrate modified peptide are then separated, and the product modified peptide is measured. The present invention is also directed to kits and bioreagents for performing the assays. Table 1 of the patent shows a list of substrates that have a fluorescent dye attached to the substrate. Shultz does not teach the substrate having two dyes attached or a library of compounds.

In the paper Ventura teaches phorbol ester regulation of opioid peptide gene expression in myocardial cells and the role of nuclear protein kinase C. Opioid peptide gene expression was characterized in adult rat ventricular cardiac myocytes that had been cultured in the absence or the presence of phorbol 12-myristate 13-acetate. The phorbol ester induced a concentration- and time-dependent increase of prodynorphin mRNA, the maximal effect being reached after 4 hours of treatment. The increase in mRNA expression was suppressed by incubation of cardiomyocytes with staurosporine, a putative protein kinase C inhibitor, and was not observed when the cells were cultured in the presence of the inactive phorbol ester 4a-phorbol 12,13-didecanoate. Incubation of cardiac myocytes with phorbol 12-myristate 13-acetate also elicited a specific and staurosporine-sensitive increase in immunoreactive dynorphin B, a biologically active end product of the precursor, both in the myocardial cells and in the culture medium. In vitro run-off transcription assays indicated that transcription of the prodynorphin gene was increased both in nuclei isolated from phorbol ester-treated myocytes and in nuclei isolated from control cells and then exposed to phorbol 12-myristate 13-acetate. No transcriptional effect was observed when cardiac myocytes or isolated nuclei where exposed to 4a-phorbol 12,13-didecanoate. The phorbol ester-induced increase in prodynorphin gene transcription was prevented by pretreatment of myocytes or isolated nuclei with staurosporine, suggesting that myocardial opioid gene expression may be regulated by nuclear protein kinase C. In this regard, cardiac myocytes expressed protein kinase C-α, -δ, -ε, and -ζ, as shown by immunoblotting. Only protein kinase C-δ and protein kinase C-ε were expressed in nuclei that have been isolated from control myocytes, suggesting that these 2 isotypes of the enzyme may be part of the signal transduction pathway involved in the effect elicited by the phorbol ester an opioid gene transcription in isolated nuclei. The incubation of myocardial nuclei isolated from control cells

in the presence of a protein kinase C activator induced the phosphorylation of the myristylated alanine-rich protein kinase C substrate peptide, a specific fluorescent substrate of the enzyme. The possibility that prodynorphin gene expression may control the heart function through autocrine or paracrine mechanisms is discussed. Ventura does not teach the substrate having two dyes attached or a library of compounds.

In the paper Blumenthal reviews the development and characterization of fluorescently-labeled myosin light chain kinase calmodulin-binding domain peptides. The development and characterization of peptides based on the sequence of the calmodulin-binding domain of skeletal muscle myosin light-chain kinase which were labeled with the fluorescent reagent, acrylodan are described. The use of these fluorescently-labeled peptides to study various aspects of calmodulin-peptide interactions including binding affinity, stoichiometry, specificity, changes in peptide conformation, and thermal stability of the peptide-calmodulin complex is demonstrated. Page 46 discusses the preparation of analogs by replacing different amino acids within a natural peptide sequence to examine these properties. Blumenthal also teaches the formation of a library of peptides with different fluorescent labels. Page 46 also discusses the change in the acrylodan fluorophore as the environment changes and how this is useful in determining various properties. The peptides exhibit many of the salient features seen with calmodulin-target enzyme interactions. The fluorescently-labeled peptides should serve as useful models for studying calmodulin-target enzyme interactions at the molecular level.

It would have been obvious to one of skill in the art at the time of the invention to incorporate a double label selected from those taught by Odom or Tyagi in the Macala, Shultz or Ventura substrates because of the ability to detect conformational changes in the substrate due to covalent modifications of the substrates as shown by Odom and Tyagi. One of skill in the art would also have recognized that libraries of substrates as taught by Blumenthal would have allowed the Macala, Shultz or Ventura substrates to be used for characterizing enzyme properties as shown by Blumenthal.

7. Applicant's arguments with respect to the claims have been considered but are moot in view of the new ground(s) of rejection. The Odom reference clearly anticipates the instant claims as outlined above and further shows that the double label has advantages over a single

label in assays that conformation changes occur. The Tyagi reference clearly shows advantages of using non-FRET label pairs.

8. The prior art made of record and not relied upon is considered pertinent to applicant's disclosure. The additionally cited art relates to fluorescently labeled substrates and their analytical use.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Arlen Soderquist whose telephone number is (571) 272-1265. The examiner's schedule is variable between the hours of about 6:30 AM to about 5:00 PM on Monday through Thursday and alternate Fridays.

A general phone number for the organization to which this application is assigned is (571) 272-1700. The fax phone number to file official papers for this application or proceeding is (703) 872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Arlen J. Soderquist

December 1, 2004

ARLEN SODERQUIST
PRIMARY EXAMINER